Remarks

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Detection of Sequence Differences

Large-scale multiplex analysis of highly polymorphic loci is needed for practical identification of individuals, e.g., for paternity testing and in forensic science, for organ-transplant donor-recipient matching, for genetic disease diagnosis, prognosis, and pre-natal counseling, and for the study of oncogenic mutations. In addition, the cost-effectiveness of infectious disease diagnosis by nucleic acid analysis varies directly with the multiplex scale in panel testing. Many of these applications depend on the discrimination of single-base differences at a multiplicity of sometimes closely space loci.

A variety of DNA hybridization techniques are available for detecting the presence of one or more selected polynucleotide sequences in a sample containing a large number of sequence regions. In a simple method, which relies on fragment capture and labeling, a fragment containing a selected sequence is captured by hybridization to an immobilized probe. The captured fragment can be labeled by hybridization to a second probe which contains a detectable reporter moiety.

Another widely used method is Southern blotting. In this method, a mixture of DNA fragments in a sample are fractionated by gel electrophoresis, then fixed on a nitrocellulose filter. By reacting the filter with one or more labeled probes under hybridization conditions, the presence of bands containing the probe sequence can be identified. The method is especially useful for identifying fragments in a restriction-enzyme DNA digest which contain a given probe sequence, and for analyzing restriction-fragment length polymorphisms ("RFLPs").

Another approach to detecting the presence of a given sequence or sequences in a polynucleotide sample involves selective amplification of the sequence(s) by polymerase chain reaction. In this method, primers complementary to opposite end portions of the selected sequence(s) are used to promote, in conjunction with thermal cycling, successive rounds of primer-initiated replication. The amplified sequence may be readily identified by a variety of techniques. This approach is particularly useful for detecting the presence of low-copy sequences in a polynucleotide-containing sample, e.g., for detecting pathogen sequences in a body-fluid sample.

More recently, methods of identifying known target sequences by probe ligation methods have been reported. In one approach, known as oligonucleotide ligation assay ("OLA"), two probes or probe elements which span a target region of interest are hybridized with the target region. Where the probe elements match (basepair with) adjacent target bases at the confronting ends of the probe elements, the two elements can be joined by ligation, e.g., by treatment with ligase. The ligated probe element is then assayed, evidencing the presence of the target sequence.

In a modification of this approach, the ligated probe elements act as a template for a pair of complementary probe elements. With continued cycles of denaturation, hybridization, and ligation in the presence of the two complementary pairs of probe elements, the target sequence is amplified geometrically, i.e., exponentially allowing very small amounts of target sequence to be detected and/or amplified. This approach is referred to as ligase chain reaction ("LCR").

Another scheme for multiplex detection of nucleic acid sequence differences is disclosed in U.S. Patent No. 5,470,705 to Grossman et. al. where sequence-specific probes, having a detectable label and a distinctive ratio of charge/translational frictional drag, can be hybridized to a target and ligated together. This technique was used in Grossman, et. al., "High-density Multiplex Detection of Nucleic Acid Sequences: Oligonucleotide Ligation Assay and Sequence-coded Separation," Nucl. Acids Res. 22(21):4527-34 (1994) for the large scale multiplex analysis of the cystic fibrosis transmembrane regulator gene.

Jou, et. al., "Deletion Detection in Dystrophin Gene by Multiplex Gap Ligase Chain Reaction and Immunochromatographic Strip Technology," Human Mutation 5:86-93 (1995) relates to the use of a so called "gap ligase chain reaction" process to amplify simultaneously selected regions of multiple exons with the amplified products being read on an immunochromatographic strip having antibodies specific to the different haptens on the probes for each exon.

There is a growing need, e.g., in the field of genetic screening, for methods useful in detecting the presence or absence of each of a large number of sequences in a target polynucleotide. For example, as many as 400 different mutations have been associated with cystic fibrosis. In screening for genetic predisposition to this disease, it is optimal to test all of the possible different gene sequence mutations in the subject's genomic DNA, in order to make a positive identification of "cystic fibrosis". It would be ideal to test for the presence or absence of all of the possible mutation sites in a single assay. However, the prior-art methods described above are not readily adaptable for use in detecting multiple selected sequences in a convenient, automated single-assay format.

Solid-phase hybridization assays require multiple liquid-handling steps, and some incubation and wash temperatures must be carefully controlled to keep the stringency needed for single-nucleotide mismatch discrimination. Multiplexing of this approach has proven difficult as optimal hybridization conditions vary greatly among probe sequences.

Allele-specific PCR products generally have the same size, and a given amplification tube is scored by the presence or absence of the product band in the gel lane associated with each reaction tube. This approach requires splitting the test sample among multiple reaction tubes with different primer combinations, multiplying assay cost. PCR has also discriminated alleles by attaching different fluorescent dyes to competing allelic primers in a single reaction tube, but this route to multiplex analysis is limited in scale by the relatively few dyes which can be spectrally resolved in an economical manner with existing instrumentation and dye chemistry. The incorporation of bases modified with bulky side chains can be used to differentiate allelic PCR products by their electrophoretic mobility, but this method is limited by the successful incorporation of these modified bases by polymerase, and by the ability of electrophoresis to resolve relatively large PCR products which differ in size by only one of these groups. Each PCR product is used to look for only a single mutation, making multiplexing difficult.

Ligation of allele-specific probes generally has used solid-phase capture or size-dependent separation to resolve the allelic signals, the latter method being limited in multiplex scale by the narrow size range of ligation probes. The gap ligase chain reaction process requires an additional step -- polymerase extension. The use of probes with distinctive ratios of charge/translational frictional drag technique to a more complex multiplex will either require longer electrophoresis times or the use of an alternate form of detection.

The need thus remains for a rapid single assay format to detect the presence or absence of multiple selected sequences in a polynucleotide sample.

Use of Oligonucleotide Arrays for Nucleic Acid Analysis

Ordered arrays of oligonucleotides immobilized on a solid support have been proposed for sequencing, sorting, isolating, and manipulating DNA. It has been recognized that hybridization of a cloned single-stranded DNA molecule to all possible oligonucleotide probes of a given length can theoretically identify the corresponding complementary DNA segments present in the molecule. In such an array, each oligonucleotide probe is

immobilized on a solid support at a different predetermined position. All the oligonucleotide segments in a DNA molecule can be surveyed with such an array.

One example of a procedure for sequencing DNA molecules using arrays of oligonucleotides is disclosed in U.S. Patent No. 5,202,231 to Drmanac, et. al. This involves application of target DNA to a solid support to which a plurality of oligonucleotides are attached. Sequences are read by hybridization of segments of the target DNA to the oligonucleotides and assembly of overlapping segments of hybridized oligonucleotides. The array utilizes all possible oligonucleotides of a certain length between 11 and 20 nucleotides, but there is little information about how this array is constructed.

WO 89/10977 to Southern discloses the use of a support carrying an array of oligonucleotides capable of undergoing a hybridization reaction for use in analyzing a nucleic acid sample for known point mutations, genomic fingerprinting, linkage analysis, and sequence determination. The matrix is formed by laying nucleotide bases in a selected pattern on the support. This reference indicates that a hydroxyl linker group can be applied to the support with the oligonucleotides being assembled by a pen plotter or by masking.

WO 94/11530 to Cantor also relates to the use of an oligonucleotide array to carry out a process of sequencing by hybridization. The oligonucleotides are duplexes having overhanging ends to which target nucleic acids bind and are then ligated to the non-overhanging portion of the duplex. The array is constructed by using streptavidin-coated filter paper which captures biotinylated oligonucleotides assembled before attachment.

WO 93/17126 to Chetverin uses sectioned, binary oligonucleotide arrays to sort and survey nucleic acids. These arrays have a constant nucleotide sequence attached to an adjacent variable nucleotide sequence, both bound to a solid support by a covalent linking moiety. The constant nucleotide sequence has a priming region to permit amplification by PCR of hybridized strands. Sorting is then carried out by hybridization to the variable region. Sequencing, isolating, sorting, and manipulating fragmented nucleic acids on these binary arrays are also disclosed. In one embodiment with enhanced sensitivity, the immobilized oligonucleotide has a shorter complementary region hybridized to it, leaving part of the oligonucleotide uncovered. The array is then subjected to hybridization conditions so that a complementary nucleic acid anneals to the immobilized oligonucleotide. DNA ligase is then used to join the shorter complementary region and the complementary nucleic acid on the array. There is little disclosure of how to prepare the arrays of oligonucleotides.

WO 92/10588 to Fodor et. al., discloses a process for sequencing, fingerprinting, and mapping nucleic acids by hybridization to an array of oligonucleotides. The array of oligonucleotides is prepared by a very large scale immobilized polymer

synthesis which permits the synthesis of large, different oligonucleotides. In this procedure, the substrate surface is functionalized and provided with a linker group by which oligonucleotides are assembled on the substrate. The regions where oligonucleotides are attached have protective groups (on the substrate or individual nucleotide subunits) which are selectively activated. Generally, this involves imaging the array with light using a mask of varying configuration so that areas exposed are deprotected. Areas which have been deprotected undergo a chemical reaction with a protected nucleotide to extend the oligonucleotide sequence where imaged. A binary masking strategy can be used to build two or more arrays at a given time. Detection involves positional localization of the region where hybridization has taken place. K. L. Beattie, et. al., "Advances in Genosensor Research," Clin. Chem. 41(5): 700-09 (1995) discloses attachment of previously assembled oligonucleotide probes to a solid support.

There are many drawbacks to the procedures for sequencing by hybridization to such arrays. Firstly, a very large number of oligonucleotides must be synthesized. Secondly, there is poor discrimination between correctly hybridized, properly matched duplexes and those which are mismatched. Finally, certain oligonucleotides will be difficult to hybridize to under standard conditions, with such oligonucleotides being capable of identification only through extensive hybridization studies.

The present invention is directed toward overcoming these deficiencies in the art.

The rejection of claims 120-125, 128, and 135-137 under 35 U.S.C. § 112 (1st para.) for failure to satisfy the written description requirement is respectfully traversed. The limitation "wherein each capture oligonucleotide probe of the array differs from its adjacent capture oligonucleotide probe by at least 25% of the nucleotides" is fully supported by original claim 81 of the present application. Section 112 makes no requirement that the entire scope of claim 81 be incorporated into claim120 in order to satisfy the written description requirement. It is beyond question that original claim 81 contains the disputed limitation and that should end any question about applicants' satisfaction of the written description requirement. In any event, this limitation is also supported on page 43, lines 22-24 and page 45, lines 35-37 of the present application. For all of these reasons, the rejection under 35 U.S.C. § 112 (1st para.) should be withdrawn.

The rejection of claims 120-125, 128, and 136-137 under 35 U.S.C. § 112 (2nd para.) for indefiniteness is respectfully traversed in view of the above amendments.

The rejection of claims 120-125 and 136-137 under 35 U.S.C. § 102(e) as anticipated by U.S. Patent No. 5,744,305 to Fodor et. al., ("Fodor") is respectfully traversed.

Fodor relates to the preparation of arrays of polymeric materials attached to a solid support using solid-phase chemistry, photolabile protecting groups, and photolithography. The polymeric materials can be assembled from any of the following monomers: L-amino acids, D-amino acids, synthetic amino acids, nucleotides, pentoses, and hexoses. No where does the outstanding office action make any effort to demonstrate how Fodor satisfies the "wherein each capture oligonucleotide probe of the array differs from its adjacent capture oligonucleotide probe by at least 25% of the nucleotides" limitation of the claims. Indeed, in Figures 1, 2, 6, 7, and 8, which do not even show oligonucleotide probes with greater than 16 nucleotides, the adjacent capture oligonucleotide probes are nearly identical. The reason for this is that the array of Fodor is predicated on changing just one base to detect differences. This is very different from the present invention where at least 25% of the nucleotides in adjacent capture probes are different. Since Fodor fails to teach or suggest the claimed invention, the rejection based on it should be withdrawn.

The rejection of claims 120-125 and 136-137 under 35 U.S.C. § 102(e) as anticipated by U.S. Patent No. 5,837,832 to Chee et. al., ("Chee") is respectfully traversed.

Chee teaches arrays of nucleic acid probes on biological chips. The outstanding office action states that SEQ ID NOs: 9 and 10 of Chee differ by more than 25% of the nucleotides. Applicants respectfully disagree. Firstly, Chee does not say that these sequences are adjacent to one another on the array. However, even if they were, they do not satisfy the "greater than sixteen nucleotides" or "wherein each capture oligonucleotide probe of the array differs from its adjacent capture oligonucleotide probe by at least 25% of the nucleotides" limitations of the claims. In particular, the oligonucleotide probes of SEQ ID NOs: 9 and 10 each actually stand for 4 different nucleotide sequences depending on what X is selected to be. Since each of the 4 SEQ ID NO: 9s and each of the 4 SEQ ID NO: 10s only differ from another by a single base, each of the 4 SEQ ID NO: 9s and each of the 4 SEQ ID NO: 10s differ from one another by less than 25%. Moreover, when properly aligned, as shown below, it is apparent that SEQ ID NOs: 9 and 10 of Chee, which have 15 nucleotides, have 12-13 identical nucleotides and differ by only 2-3 nucleotides:

TTTATAXTAGAAACC (SEQ ID NO: 9)
TTATAGXAGAAACCA (SEQ ID NO: 10)

Since X can be A, T, C, or G, SEQ ID NOs: 9 and 10 have only 2 differences if X is G in SEQ ID NO: 9 and X is T in SEQ ID NO: 10. Otherwise, there will be 3 differences between these sequences. In either event, assuming that SEQ ID NOs: 9 and 10 are adjacent to one another (which there is no evidence of), they fail to satisfy the "greater than sixteen

nucleotides" or "wherein each capture oligonucleotide probe of the array differs from its adjacent capture oligonucleotide probe by at least 25% of the nucleotides" limitations of the claims. Accordingly, the rejection based on Chee should be withdrawn.

The rejection of claims 120-125, 128, and 137-138 under the judicially-created doctrine of obviousness-type double patenting as unpatentable over U.S. Patent Application Serial No. 10/272,152 is respectfully traversed in view of applicants' submission of the accompanying terminal disclaimer.

The rejection of claims120-125, 128, and 137-138 under the judicially-created doctrine of obviousness-type double patenting as unpatentable over U.S. Patent Application Serial No. 08/794,851 ("'851 application") is respectfully traversed. During prosecution of the '851 application, the U.S. Patent and Trademark Office imposed a restriction requirement between a method of identifying a sequence/a kit for doing so (claims 1-88 and 138-147), a method of forming an array (claims 89-119), and an array (claims 120-137). A copy of the restriction requirement is attached. In the '851 application, the method of identifying a sequence/a kit for doing so was elected. Subsequently, applicants filed the present divisional application to cover array claims 120-137. In view of the imposition of a restriction requirement, 35 U.S.C. § 121 precludes making a double patenting rejection. Accordingly, the double patenting rejection based on the '851 application should be withdrawn.

The rejection of claims 120-125, 128, and 137-138 under the judicially-created doctrine of obviousness-type double patenting as unpatentable over U.S. Patent No. 6,506,594 is respectfully traversed in view of applicants' submission of the accompanying terminal disclaimer.

In view of all the foregoing, it is submitted that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: August 13, 2004

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